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Global Population Structure of the Dusky Shark and Geographic Sourcing of Shark Fins from Commercial Markets

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By

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Abstract

The dusky shark, *Carcharinus obscurus*, is a globally distributed, coastal-pelagic species subject to an apparent high level of exploitation. The International Union for the Conservation of Nature (IUCN) lists this species as "Vulnerable" globally, and "Endangered" within western North Atlantic and Gulf of Mexico waters due to an over 80% decline in this region, with no evidence of population recovery. The extensive exploitation of dusky sharks may partly be attributed to the high market value of its fins, but the contribution of individual dusky shark stocks to the fin markets is unknown. This knowledge would be helpful to detect if specific stocks are experiencing disproportionate levels of exploitation. Due to its susceptibility to overfishing, current dire conservation status and need for additional information on its population dynamics, we analyzed the genetic population structure and genetic diversity of the dusky shark (n = 415) across 8 globally distributed locations utilizing 10 nuclear microsatellite loci. The nuclear marker analyses support and extend previously published mitochondrial marker work, identifying a strong divergence among Atlantic and Indo-Pacific samples. Furthermore, nuclear marker results indicate the presence of six genetically discrete management units for dusky sharks, with significant genetic differentiation between the western North Atlantic, South African, and each of three Australian site collections (N, E and W coasts). Discovery of these nuclear microsatellite-defined, smaller geographic scale management units provides a basis for the assignment of market-derived fins to their population of origin with the use of genetic assignment techniques.

Key Words: genetic population structure, sharks, management, conservation, genetic assignment

Introduction

Fisheries dependent data have revealed that extensive declines of many shark species are occurring globally (Castro *et al.* 1999; Myers and Worm 2003; Baum *et al.* 2003; Pauly *et al.* 1998; Myers *et al.* 2007). Although these reductions have been attributed to multiple factors, high exploitation levels due to the strong market demand for shark fins has been identified as one of the key elements contributing to these declines (Castro *et al.* 1999). To curb the ever-increasing exploitation of sharks, the United Nations Food and Agriculture Organization (FAO) implemented an International Plan of Action (IPOA) for the Conservation and Management of Sharks in 1999, which encouraged independent nations to develop management plans and rigorously monitor shark exploitation rates at a species-specific level (FAO 1999). Since implementation of the IPOA in 2000, a 20% decrease in catches have been documented among the 26 countries reporting the highest rates of exploitation (Fischer *et al.* 2012); however, incomplete- (poor species-level catch records) or under-reporting (bycatch discards, illegal finning practices, and non-reported artisanal harvests) of catches to the FAO remains pervasive among some shark fishers and nations, introducing much uncertainty into the population status of numerous shark species (Worm *et al.* 2013). Further compounding the efforts to manage legally exploited species is the illegal trade of shark fins and other products. Although the illegal trade of wildlife products is regulated internationally by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), many nations lack the infrastructure and logistical abilities required to prevent restricted wildlife products from entering commercial markets (Ferrier 2009). Thus, many species of shark protected by CITES legislation, including *Carcharodon carcharias* (Shivji *et al.* 2005), *Cetorhinus*

maximus (Magnussen *et al.* 2007), *Sphyrna mokarran* (Abercrombie *et al.* 2005) and *Carcharhinus longimanus* (Clarke *et al.* 2006b), remain targets of the shark fin trade due to their high market value, despite national and international harvest moratoria and strict trade regulations. Further aggravating management efforts is an overall lack of biological data concerning the population biology and ecology of elasmobranchs (Lack and Sant 2009; Dulvy *et al.* 2008; Pilling *et al.* 2009), including data pertaining to their genetic population structure across both broad and fine geographic scales. This paucity of data concerning the fundamental biology and connectivity of sharks make it nearly impossible to effectively manage exploited populations and to monitor the illegal and legal trade of shark fins and their other body parts.

Efforts to monitor the harvest and trade of sharks will benefit from the delineation of genetic management units (MUs; Moritz 1994), as genetic assignment techniques have been previously applied to help geographically source wildlife products where MUs were sufficiently genetically differentiated. For instance, such methods have been employed to geographically source illegally traded and/or harvested African elephant ivory (Wasser *et al.* 2004), European pond turtles (Velo-Antón *et al.* 2007), and Norwegian minke whale products (Glover *et al.* 2012), but have yet to be used to source market-derived shark fins (Shivji 2010). As harvested shark fins are often sold as unlabeled and processed materials, determining the species composition of market fins and/or products may be extremely difficult; however, researchers have recently applied genetic tools to perform species identification of Hong Kong market-derived fins with high success (Clarke *et al.* 2006a). While species identification is a critical first step towards proper monitoring of the fin trade, identification of the specific geographic regions subject to high market

exploitation is also vital to ensure sustainable fishing practices within discrete MUs. The Hong Kong fin market imports shark fins harvested from every ocean basin which includes national waters from more than 80 countries worldwide (Clarke 2004); however, the monitoring of geographically localized, species-specific exploitation rates is not currently feasible due to incomplete trade records. Thus, genetic assignment techniques may provide a vital means to identify the geographic origin of market derived shark fins, and also present existing fisheries management systems with a more accurate estimate of unreported and/or illegal fishing within exploited MUs.

The dusky shark, *Carcharinus obscurus*, is a globally distributed and highly exploited coastal-pelagic species that inhabits warm temperate and tropical waters (Musick *et al.* 2009). The innate reproductive biology of this species makes it especially vulnerable to exploitation as dusky sharks are slow growing and among the latest to mature of all shark species (17-20 years). In addition, females give birth to small litters (3-12; Romine *et al.* 2009) and possess a three year reproductive cycle (Last and Stevens 1994; Natanson *et al.* 1995; Romine *et al.* 2009; Simpfendorfer *et al.* 2002; Cortés 1998; Smith *et al.* 1998). Combined, these reproductive traits ensure that dusky sharks possess one of the lowest population rebound potentials among all members of the family Carcharhinidae (Smith *et al.* 1998). Exploitation of the dusky shark occurs globally, consisting of artisanal (Castillo-Géniz *et al.* 1998; Blaber *et al.* 2009; Pérez-Jiménez *et al.* 2005), recreational (Heald 1987; Simpfendorfer 1999; Simpfendorfer and Donohue 1998), commercial, and industrial fishing efforts (Mazzoleni and Schwingel 1999), which notably includes a high by-catch mortality as a result of pelagic longline fishing for tunas and swordfish (Cortés *et al.* 2006). The demand for dusky shark fins in the Asian market

is at least partially responsible for such high rates of global exploitation. Estimates based on trade records and genetic identification of fins from commercial markets indicate that dusky sharks comprised ~ 1.4% of total fins auctioned in 2000, which represents an annual harvest of between 150,000 and 750,000 individuals, occurring exclusively for the fin trade (Clarke *et al.* 2006a, eb).

Such intense fishing pressure has led to severe reductions in many regions across the dusky shark's global distribution (Musick *et al.* 2009). Within the waters of the western North Atlantic and Gulf of Mexico, depletions of greater than 80% of the dusky shark's virgin biomass has been estimated (Cortés *et al.* 2006). Furthermore, reductions in neonate recruitment within southwest Australian waters suggest that a decline in reproductively mature sharks has also occurred within this region as a result of a high rate of fishing mortality (Rogers *et al.* 2013a; McAuley *et al.* 2007). Such widespread declines have prompted several key conservation actions. The International Union for the Conservation of Nature (IUCN) Red List of Threatened Species has listed the dusky shark as globally 'Vulnerable' and accordingly, some nations have instituted federal restrictions on catches within their waters, for instance: (i) in 2005 the Australian government issued a number of federal regulations, including gear restrictions, seasonal area closures, and maximum size limits for dusky sharks (McAuley 2008; McAuley *et al.* 2005); and (ii) in 2000 the United States government implemented a moratorium on landings of dusky sharks within all US federal waters (Musick *et al.* 2009). Yet, despite this moratorium, declines are still occurring within the western North Atlantic and Gulf of Mexico due to unreported fishing outside federal waters, and incidental by-catch (Cortés *et al.* 2006; SEDAR 2011; Romine *et al.* 2009), underscoring the need for more

rigorous cross-jurisdictional management of this species. However, presently little is known regarding the genetic population structure of this species and the number of global genetic management units that currently exist.

Previous analysis of dusky shark maternally inherited mitochondrial DNA (mtDNA) has revealed both broad- and fine-scale genetic population structure across this species' surveyed range. Benavides *et al.* (2011) identified (at least) three distinct globally distributed matrilineal MUs, which consisted of: (i) the western North Atlantic, (ii) South Africa, and (iii) Australia (eastern and western), while Geraghty *et al.* (2014) and Ovenden *et al.* (2009) provided evidence for fine-scale genetic population structure within the Indo-Australian archipelago. As mitochondrial DNA does not undergo recombination and is maternally inherited in vertebrates (Wilson *et al.* 1985; Avise *et al.* 1987), defining connectivity based solely on a single mitochondrial locus can be misleading. The lack of recombination within the haploid mitogenome allows mtDNA to be a highly effective evolutionary tool to define long standing evolutionary lineages, however, hyper-variable diploid markers that readily undergo recombination may be more applicable to identifying contemporary patterns of genetic connectivity (Wilson *et al.* 1985). Furthermore, maternal inheritance of the mitogenome allows only for the reconstruction of maternal lineages, and therefore provides no information regarding male-mediated connectivity patterns (Wilson *et al.* 1985; Avise *et al.* 1987).

To extend previous mitochondrial DNA work and provide a more comprehensive view of the dusky shark's global genetic connectivity, genetic diversity and population demographics, I utilize bi-parentally inherited nuclear microsatellite genetic markers to address these issues. Furthermore, I use the resulting information on genetic connectivity

to Hong Kong fin markets to determine the relative contribution of each of the defined populations to the markets.

Methods

Tissue Sampling, DNA Extraction, and Genotyping Conditions

A total of 415 *C. obscurus* tissue samples were collected from 8 globally distributed sampling locations: US east coast (*n* = 99), US Gulf of Mexico (*n* = 11), Brazil (*n* = 6), South Africa (*n* = 70), Indonesia (*n* = 16), Western Australia (*n* = 99), Eastern Australia (*n* = 84), and Northern Australia (*n* = 33). All tissue samples were preserved in 95% ethanol or DMSO (Indonesia) until genomic DNA extraction. Genomic DNA (gDNA) was extracted from approximately 25mg of tissue using the DNeasy® Blood and Tissue Kit (Qiagen Inc., Valencia, CA). A subset of the individuals utilized in this study was also used by Benavides *et al.* (2011): Eastern Australia (*n* = 16), Western Australia (*n* = 35), US east coast (*n* = 76), and South Africa (*n* = 47); as well as Geraghty *et al.* (2014): Eastern Australia (*n* = 70), Indonesia (*n* = 16), Northern Australia (*n* = 33). The exact capture locations of *C. obscurus* individuals caught within Indonesian waters are unknown, as these samples were sampled opportunistically from a market (Tanjung Luar) located in eastern Lombok (Geraghty *et al.* 2014).

All sampled *C. obscurus* were cross-amplified and genotyped at a total of ten polymorphic microsatellite loci originally isolated from four other species of shark: *C. limbatus*, *C. perezi*, *C. tilstoni*, and *Prionace glauca* (Table 1). Each microsatellite locus was amplified in a total polymerase chain reaction (PCR) volume of 25µl and contained 1µl of unquantified genomic DNA, 1X PCR buffer (1.5 mM $MgCl_2$), 0.2mM of each

dNTP, 0.33-0.4mM $MgCl_2$, 0.5U of HotStar *Taq*™ DNA Polymerase (Qiagen Inc.), 0.16-0.20μM of the corresponding Forward primer with the associated 5'-M13 tail (Schuelke 2000), and 0.4μM of the fluorescently labeled universal M13 primer (5'-TGTAAAACGACGGCCAGT-3') and Reverse primer. The forward primer of locus *Pgla-02* was fluorescently labeled and therefore PCR reactions of this locus did not contain a fluorescently labeled M13 primer. Optimum annealing temperatures and magnesium concentrations were determined independently for each locus (Table 1). PCR thermal profiles were as follows: 95°C initial heating for 15 minutes (min), followed by 35 cycles of 94°C for 1 min, 1 min at the locus-specific primer annealing temperature (Table 1), 72°C for 1 min, and a final 20 min extension step at 72°C. Each locus was amplified independently and then subsequently combined for fragment electrophoresis according to amplicon size and fluorescent label color (Table 1). Electrophoresis was performed using an automated AB 3130 genetic analyzer and all fragments were sized using LIZ 600 and the software Genemapper v.3.7 (Applied Biosystems Inc., Foster City, CA).

Table 1. Genetic characterization of ten nuclear microsatellite loci used to survey variation in the dusky shark (*Carcharhinus obscurus*). Abbreviations: size range: fragment size of amplified microsatellite in base pairs (bp); *N*, number of alleles; T_a, annealing temperature; MgCl (mM), magnesium chloride concentration per reaction volume.

Locus	**Source**	**Size range (bp)**	***N***	***T_a* (°C)**	**MgCl**	**M13 fluorescent dye**	**Repeat Motif**
Cpe-242	Horn *et al.* (unpublished)	231-245	7	60	0.33	VIC	$(CA)_3GA(CA)_{10}$
Cpe-276	Horn *et al.* (unpublished)	192-260	36	60	0.45	6-FAM	$(AG)_{25}$
Cpe-352	Horn *et al.* (unpublished)	124-156	15	60	0.33	6-FAM	$(CA)_{15}$
Cpe-421	Horn *et al.* (unpublished)	116-170	29	58	0.45	NED	$(CA)_{15}$
Cli-107	Keeny and Heist 2008	116-132	9	60	0.33	VIC	$(GT)_{14}$
Cli-108	Keeny and Heist 2008	135-145	6	58	0.33	VIC	$(GT)_{12}$
Ct-06	Ovenden *et al.* 2006	265-399	22	58	0.33	NED	$(CA)_{14}$
Pgla-1	Fitzpatrick *et al.* 2011	227-245	7	60	0.33	PET	$(TCC)_7(TCC)_3TCG(TCC)_5$
Pgla-2	Fitzpatrick *et al.* 2011	125-149	10	60	0.4	*6-FAM	$(TCC)_5TCG(TCC)_2$ $(TCG)_2$
Pgla-5	Fitzpatrick *et al.* 2011	171-231	20	60	0.33	NED	$(GT)_{27}(GA)_{19}$

* Indicates fluorescently labeled primer

Summary Statistics: Genetic Variation, Hardy-Weinberg, and Linkage Equilibrium

The number of alleles per locus (*a*), allelic size range (*as*), and levels of observed (H_O) and expected (H_E) heterozygosity were estimated using GENEPOP on the web 3.4 (Raymond and Rousset 1995; Rousset 2008). Exact tests, as implemented in GENEPOP, were used to test for departures from Hardy-Weinberg equilibrium (HWE) within collections and across the global sample set as well as for linkage disequilibrium (LD) between pairs of loci. Significance of exact tests was computed using a Markov chain algorithm, as implemented in GENEPOP (Guo and Thompson 1992) (dememorization 1000; batches 100; iterations per batch 1000). *P*-values were adjusted using a sequential Bonferroni correction where multiple comparisons were made (Rice 1989). Allelic richness (R_S) (El Mousadik and Petit 1996), standardized by sample size, and gene diversity (*Gd*) (Nei 1987), weighted by regional sample size, were calculated using FSTAT 2.9 (Goudet 2001). Locations with low sample sizes were omitted from R_S and *Gd* calculations as the inclusion of such sample sites would allow for little if any inference of the relative genetic diversity among sites. Variation in genetic diversity (R_S and *Gd*) among capture locations was tested for significance using a Kruskal-Wallis one-way analysis of variance, as implemented in the software JMP 10.0.02 (SAS Institute, Cary, NC).

The program FreeNA (Chapuis and Estoup 2007) was used to estimate the frequency of null alleles, and calculate null allele-corrected estimates of pairwise F_{ST}, to assess the effects of detected levels of null alleles (see Results) on estimates of population differentiation. The program POWSIM (Ryman and Palm 2006) was used to evaluate the combined statistical power of the set of microsatellite markers and sampling regime

utilized in the present study to infer genetic differentiation (Chi-square and Fisher's exact test). POWSIM was performed using 1000 dememorizations, 100 batches, and 1000 iterations per batch.

The majority (~66%) of the *C. obscurus* sampled for this study were classified as immature [total length (TL) was less than the estimated size at maturity [females: 220-250cm; males: 230-243cm (Simpfendorfer *et al.* 2002)] (Table 2). As immature dusky sharks may possess prolonged residency periods within nursery areas (Dudley *et al.* 2005), biologically related individuals may have been inadvertently sampled. Unobserved family structure may amplify any allele frequency differences among groups (Falush *et al.* 2003); thus, to avoid any potential bias occurring from the sampling of biologically related individuals, the genetic relatedness of individuals within sampling locations was determined. Pairwise coefficients of relatedness were estimated using a maximum likelihood estimation method as implemented in the program ML-RELATE (Kalinowski *et al.* 2006). ML-RELATE was used to categorize the relationship between pairs of individuals as full-siblings, half-siblings, parent-offspring, or as unrelated.

Table 2. Number of mature and immature male and female *C. obscurus* individuals captured within each sampling location based on total length (TL) measurements. Mature: TL>220cm females, >230cm males, and immature: TL: <220cm females, <230cm males. Unknown: individuals lacking sex and TL data.

		Males		Females		Unknown
	n	Immature	Mature	Immature	Mature	
US East Coast	95	43	1	36		15
Gulf of Mexico	11					11
Brazil	6					6
South Africa	70	10		9	1	50
West Australia	99	35		22	4	38
East Australia	84	4	24	12	25	19
North Australia	33		14		17	2
Indonesia	17					17

The conversion programs Microsatellite Toolkit (Park 2001), CREATE (Coombs *et al.* 2008), and CONVERT (Glaubitz 2004) were used to generate input files for the analysis programs used in this study.

Population-level Subdivision

Pairwise population-level F_{ST} values (Weir and Cockerham 1984) were estimated using ARLEQUIN (Excoffier and Schneider 2005) (significance estimated using 10 000 permutations). Differentiation was also estimated using a secondary estimator, Jost's (2008) measure of D_{est}, utilizing the package DEMEtics (Gerlach *et al.* 2010), as implemented within the statistical software R v2.15.2 (RCoreTeam 2012). Similarly, Arlequin 3.1 (Excoffier and Schneider 2005) was used to perform a locus-by-locus hierarchical analysis of molecular variance (AMOVA) (10 000 iterations) to investigate population- and ocean basin-level divergence.

To assess if a correlation between genetic [F_{ST} / (1-F_{ST})] and geographic distance existed among *a priori* defined populations, mantel tests, as implemented in the program Isolation by Distance Web Service 3.16 (IBDWS) (Jensen *et al.* 2005) were utilized. The geographic distance among *a priori* defined sampling locations was measured as the shortest distance by sea using Google Earth (http://www.google.com/earth). Mantel tests were conducted on the global dataset (all sampling locations), as well as in a hierarchical fashion, i.e., within ocean basins (western Atlantic and Indo-Pacific). The significance of each Mantel test was assessed using 10 000 randomizations.

A multivariate approach was also used to visualize the genetic relationships among *a priori* defined populations using a Principal Coordinate Analysis (PCoA) using

the program GenAlEx 6.4 (Peakall and Smouse 2006). PCoA was performed using a covariance-standardized genetic distance measure of F_{ST} as implemented in GenAlEx 6.4 (Peakall and Smouse 2006) and allows for a graphical description of the genetic divergence among populations in multivariate space.

Individual-level Subdivision

To supplement the above population-level analyses, three individual-based clustering analyses were performed to help resolve population genetic structure: STRUCTURE 2.3.3 (Pritchard *et al.* 2000), FLOCK v2.0 (Duchesne and Turgeon 2009), and PCoA. Given the migratory nature of the dusky shark (Hussey *et al.* 2009; Davies and Joubert 1966; Kohler *et al.* 1998a), individual-based analyses may allow for the identification of genetically discrete clusters of individuals that do not correspond directly with *a priori* sampling locations.

The individual-based Bayesian clustering analysis software STRUCTURE 2.3.3 (Pritchard *et al.* 2000) was used to identify the most likely number of genetically discrete groupings using the entire global dataset. Default parameters were used for initial runs with *K*-values set ranging from 1 to 10, assuming the correlated allele frequency (Falush *et al.* 2003) and admixture models. A second STRUCTURE analysis was performed implementing Hubisz *et al.*'*s* (2009) *loc prior* model (and default settings). The *loc prior* model utilizes *a priori* sampling location information when estimating ln Pr ($X|K$), and places much more weight on clustering outcomes that are correlated with these *a priori* defined locations to better resolve differentiation where weak levels of divergence exist. For each model set, analyses were performed using ten replicates for each *K* value; each

replicate consisted of 100 000 steps of burn-in followed by 300 000 Markov chain Monte Carlo (MCMC) iterations. Results from the program STRUCTURE were analyzed using the program Structure Harvester (Earl and vonHoldt 2012) and the most likely number of *C. obscurus* populations (*K*), was determined based on two distinct criteria: (i) the value of *K* corresponding to the largest maximum posterior probability, Pr(*X/K*), and (ii) $\triangle K$, the maximum second order rate of change of Pr(*X/K*) standardized by the standard deviation of Pr(*X/K*) (Evanno *et al.* 2005). Graphical outputs from the STRUCTURE analyses were generated using DISTRUCT v 1.1 (Rosenberg 2004).

In addition to the individual-based STRUCTURE analyses, the program FLOCK v2.0 (Duchesne and Turgeon 2009) was also utilized to resolve population structure. Instead of a MCMC framework (STRUCTURE), FLOCK implements an iterative reallocation procedure to randomly allocate individual multilocus genotypes into *k*-reference, or hypothetical, populations. A range of values of *k* are set by the user and for each value of *k* the iterative reallocation procedure is performed. The FLOCK procedure requires three steps: (1) randomly allocating samples into *k* number of partitions, (2) estimating allele frequencies for each partition and reallocating individuals to the reference group where an individual's multilocus genotype likelihood score is the highest, and (3) the likelihood calculations as per the maximum likelihood method of Paetkau *et al.* (1995) (Duchesne and Turgeon 2012). This procedure is repeated for a set number of iterations within a single run and multiple replicate runs are performed for each value of *k*. To identify the most likely number of partitions, FLOCK utilizes a 'plateau analysis'. At the conclusion of each run, the mean log likelihood difference (LLOD) is estimated across all genotypes, where the LLOD corresponds to the difference

between the log likelihood of the most likely reference population and the second most likely reference population for a particular genotype. Mean LLOD scores that are identical across runs correspond to identical partitions of samples. A plateau of LLOD scores occurs when identical mean LLOD scores are generated across runs. A "plateau record" is generated based on mean LLOD values for each run and FLOCK repeats this process for each value of *k* until one of the two stopping conditions are met: (1) a single plateau of length >6 is generated, or (2) four successive values of *k* are investigated with no plateau. In the present study, plateau analysis as described by Duchesne and Turgeon (2012) was performed on a range of *k*-partitions (*k* = 2-10) using default parameters.

And finally, a multivariate approach was also used to visualize the genetic relationships among individuals with a PCoA using the program GenAlEx 6.4. The individual-level PCoA was performed using a covariance-standardized genetic distance measure of F_{ST} as implemented in GenAlEx 6.4.

Contemporary and Historical Demographic History of Species

Three distinct programs were adopted to resolve the contemporary demographic history of the dusky shark: BOTTLENECK 1.2.02 (Piry *et al.* 1999), *M*-Ratio(Garza and Williamson 2001), KGTESTS (Bilgin 2007). The program MSVAR 1.3 (Storz and Beaumont 2002) was also used to investigate historical demographic changes. Due to the minimum sample size requirements for the program BOTTLENECK, only those locations with greater than 30 individuals sampled per site were analyzed: US east coast, South Africa, Western Australia, Northern Australia, and Eastern Australia.

To identify recent population declines, the programs BOTTLENECK and *M*-Ratio were utilized. BOTTLENECK serves to identify recent population declines (within 0.2-4N_e generations, where N_e is the effective population size) by comparing estimated values of allelic diversity to the expected levels of heterozygosity (Luikart and Cornuet 1998). Assuming mutation-drift equilibrium, populations that have recently undergone a reduction in effective size are more likely to exhibit a disproportionate reduction of allelic diversity relative to the expected level of heterozygosity at polymorphic loci. Hence, a recently bottlenecked population will exhibit an excess in heterozygosity relative to the observed number of alleles within a sampled population. Significance of the allelic diversity-heterozygsoity deviation was assessed using a two-tailed Wilcoxon signed rank test (Cornuet and Luikart 1996; Luikart and Cornuet 1998), as implemented in BOTTLENECK, assuming the Two Phase Mutation (TPM) model [TPM parameters = 90% Stepwise Mutation Model (SMM), and a variance in mutational lengths of 12%].

Garza and Williamson's (2001) *M*-ratio was also used to identify population size reductions. To infer population declines, the *M*-Ratio test calculates the ratio of the number of alleles present at a given locus, *k*, to the range in allele sizes (in base pairs), *r*, so that $M = k/r$ (Garza and Williamson 2001). Following a population reduction, the ratio k/r will be reduced, as rare or low frequency alleles will likely be lost quickly, thereby reducing '*k*', whereas the range in allele size classes ('*r*') will be reduced at a much slower rate (Garza and Williamson 2001). Empirical values of *M* are then compared to critical values (M_c), which are generated by simulating equilibrium distributions of pre-bottleneck population size. To infer declines for the dusky shark, multiple critical values (M_c) were computed using four estimates of pre-bottleneck

population size [theta (θ) $=4N_e\mu$, where N_e = effective population size, and μ = the per generation mutation rate], (0.01, 0.1, 1, and 10), with the proportion of single-step mutations (p_s), and average size of non-stepwise mutations ($\triangle_g$) held constant at 0.2 and 3.5, respectively. A significant reduction in population size would be inferred if $M_c < M$.

To test for recent population expansions, the within-locus *k*- and interlocus *g*-tests (Reich *et al.* 1999), as implemented in the Excel add-in 'KGTESTS' (Bilgin 2007), were performed. These tests evaluate the variance and the distribution of allele frequencies within a population to infer demographic changes. The within-locus *k* test exploits differences in the distribution of allelic size lengths present in expanding (unimodal with high 'peakedness' or kurtosis) versus constant sized (multimodal) populations to identify population increases (Reich *et al.* 1999). The modality and kurtosis of each *a priori* defined population was evaluated using the *k*-test; significance was determined using a binomial distribution with the probability of a positive *k*-value set at its lower boundary, 0.515 as recommended by Reich *et al.* (1999). Similarly, the *g*-test exploits differences in the width of allelic distributions; expanding populations have lower inter-locus variation in allele ranges compared to stable populations (Reich *et al.* 1999). Thus, a population that is of constant size will have a higher variation across loci in allele sizes than an expanding population. Significance levels for the *g*-test are evaluated using a table of 5^{th} percentile cut-off values ($P < 0.05$), defined by the sample size and number of loci utilized (Reich *et al.* 1999). The *k*- and *g*-tests possess variable temporal sensitivities: the *k*-test is sensitive to an expansion in population size (N_e) occurring within the last 5.1 N_e generations, while the interlocus *g*-test possesses a much broader temporal sensitivity and

is better suited to identify population expansion occurring within the last 14.6 N_e generations (Reich *et al.* 1999).

The program MSVAR (Storz and Beaumont 2002) was used to investigate historical changes in effective population size. MSVAR implements a coalescent-based Bayesian likelihood analysis to generate estimates of the posterior distribution of the model parameters: N_0 (current population size), N_1 (ancestral population size), μ (mean mutation rate of all loci), and t (time since population size change). MSVAR assumes a stepwise mutational model, closed populations and conformation of populations and loci to HWE (Girod *et al.* 2011). MSVAR was run implementing the demographic model assuming exponential (rather than linear) change in population size. Each run consisted of a burn-in period of 25 000 iterations, followed by 50 000 recorded iterations (sampling every 50 000 iterations). Convergence was assessed using the Gelman-Rubin statistic using the package CODA (Plummer *et al.* 2006), as implemented in software R (RCoreTeam 2012). The final 25 000 iterations were combined across all five runs and the median and 95% posterior probability distributions were calculated for each parameter using CODA.

Geographic sourcing of market fins

As the level of genetic population structure found across the dusky shark's surveyed distribution was quite high (see results: *Population-level Subdivision*), multiple population assignment techniques were applied to genetically assign market derived *C. obscurus* fins obtained as part of our earlier studies (see Clarke *et al*. 2006a,b) to their previously unknown geographic capture site. A total of 22 fins were collected from 11

Hong Kong fin traders between November 2000 and February 2002. The 22 Hong Kong fins were originally sampled and genetically identified to species by Clarke *et al.* (2006b). All 22 Hong Kong fins were genotyped at the ten surveyed microsatellite loci used herein, and subsequently assigned to their most likely population or region of origin by utilizing the multilocus genotypes of the previously genotyped sample set (n = 415) as a baseline of the global distribution of dusky shark allele frequencies. For consistency between methods, all assignments were performed using a conservative approach by combining the reference data into defined clusters that correspond to STRUCTURE's value of K associated with $\triangle K$, the maximum value of the second order rate of change of Pr(X/K) standardized by the standard deviation of Pr(X/K) for the global dataset (see Results). Genetic assignments were performed using five distinct programs: STRUCTURE, SCAT (Wasser *et al.* 2004), ONCOR (Kalinowski *et al.* 2007), FLOCK, and GENECLASS 2.0 (Piry *et al.* 2004). Results from all five programs were compared for consistency of assignment outcomes.

Genetic assignment of the Hong Kong fins using the program STRUCTURE was performed by executing a replicate analysis (as performed above) combining the entire previously analyzed global dataset (hereafter referred to as the reference dataset) as well as the 22 genotyped Hong Kong fins. Ten replicate runs of STRUCTURE were performed utilizing the *loc prior* model, which incorporated prior sampling location information (for the reference dataset only), and assuming no admixture. Hong Kong fins were assigned to their most likely population of origin using its individual membership coefficient (q). Membership coefficients were determined for each fin by estimating the mean coefficient across all ten replicates. The program SCAT (Smoothed and Continuous

AssignmenTs) was also used to perform genetic assignments of the Hong Kong shark fins. To perform assignments, the program SCAT utilizes the allele frequencies and geographic coordinates of reference samples to create a geographic map of genetic variation (i.e., the distribution of allele frequencies) across the sampled regions using a spatial smoothing method (Wasser *et al.* 2004). For each individual to be assigned, SCAT utilizes a MCMC algorithm to generate a posterior probability distribution of its geographic origin using its multilocus genotype and the reference map. To ensure convergence of the MCMC algorithm, the analysis was replicated three times implementing 100 000 MCMC iterations. In all instances, the first 1000 iterations were discarded as burn-in, and sampling of the chain was performed every 100 iterations. Assignment scores were generated by dividing the probability of an individual's genotype originating from a given population (i.e., western Atlantic or Indo-Pacific) by the sum of all probability values for that individual as per Dellicour *et al.* (2011).

Hong Kong fin assignments were also performed using the program ONCOR. ONCOR utilizes the likelihood method of Rannala and Mountain (1997) to assign individuals, based on their multilocus genotype, to the reference population possessing the highest probability of having produced the individual's genotype.

The iterative allocation method carried out in FLOCK (Duchesne and Turgeon 2009) was also used to assign the Hong Kong fins into their most likely source population. Genetic assignment of the Hong Kong fins using the program FLOCK was performed by executing a replicate analysis (as performed above) combining the reference dataset with the 22 genotyped Hong Kong fins. Assignment scores were generated by dividing the likelihood of an individual's genotype originating from a given

population (i.e., western Atlantic or Indo-Pacific) by the sum of all likelihood values for that individual as per Dellicour *et al.* (2011).

The program GENECLASS 2.0 (Piry *et al.* 2004) was also utilized to perform genetic assignment of the Hong Kong fins. In contrast to the other assignment methods, GENECLASS' analytical framework allows for the potential that not all putative reference populations have been surveyed, and performs what is known as a 'genetic exclusion test' (Cornuet *et al.* 1999). For all GENECLASS analyses, assignment tests were performed using the partial Bayesian method (Rannala and Mountain 1997). Each fin was assigned a probability of originating from each of the reference populations (as defined by the initial STRUCTURE analyses) using the Monte Carlo method (Paetkau *et al.* 2004) to simulate 10 000 independent multilocus genotypes for each candidate population.

Results

Summary Statistics: Genetic Variation, Hardy-Weinberg, and Linkage Equilibrium

Summary statistics, including: sample size, observed (H_O) and expected (H_E) heterozygosity, average number of alleles (*a*), R_S, *Gd*, and significance of each HWE test, is provided in Table 3 for each microsatellite locus and each *a priori* defined population. The total number of alleles per locus ranged from 6 to 36. Estimated levels of H_E and H_O across all loci and populations ranged from 0.334 to 0.895 and 0.364 to 0.839, respectively. Overall, significant departures from HWE were found within all sampling locations, with the exception of the Gulf of Mexico and Brazil, after sequential Bonferroni correction ($\alpha = 0.05/70$). These departures from equilibrium were largely due

to a heterozygote deficiency at seven of the ten surveyed loci; six loci showing significant deviations in several, but not across all, *a priori* populations: Cpe-242 (US East Coast, South Africa, Western Australia, Indonesia, Northern Australia), Cpe- 276 (US East Coast, South Africa, Western Australia, Indonesia), Cpe-352 (Indonesia, Northern Australia), Cpe-421 (US East Coast, South Africa, Western Australia, Eastern Australia, Northern Australia), Pgla-1 (Eastern Australia), Pg-5 (Western Australia, Eastern Australia, Indonesia), and Ct-06 (US East Coast, South Africa, Western Australia, Eastern Australia, Indonesia, Northern Australia).

Table 3. Population-level summary statistics for each microsatellite locus and collection site for *C. obscurus*. *n*, sample size; *a*, number of alleles; R_S, allelic richness; *as*, allelic range; H_E, Nei's (1987) unbiased gene diversity, H_O, observed heterozygosity; *P*-value of Hardy-Weinberg equilibrium test; *r*, null alleles; *Gd*, gene diversity. Dash (-) indicates that the test was not performed due to low sample sizes ($n < 30$).

	Locus										
Location	Cli-107	Cli-108	Cpe-242	Cpe-276	Cpe-352	Cpe-421	Pgla-1	Pgla-2	Pgla5	Ct-06	Average across loci
US East Coast											
n	95	95	95	95	95	94	95	94	95	95	
a	7	5	3	28	6	28	10	9	8	20	12.400
R_S	2.262	1.95	2.046	5.024	2.182	5.21	2.916	3.386	2.447	4.995	9.125
as	116-130	135-143	231-243	192-260	136-154	124-180	230-245	125-149	187-203	357-399	
H_E	0.488	0.341	0.452	0.926	0.519	0.942	0.664	0.751	0.474	0.925	0.645
H_O	0.495	0.389	0.326	0.726	0.547	0.766	0.705	0.787	0.505	0.789	0.605
HW	0.849	0.496	0.005**	0.000**	0.728	0.000**	0.333	0.708	0.534	0.000*	0.000**
r	0.000	0.000	0.096	0.098	0.000	0.086	0.003	0.000	0.013	0.066	0.036
Overall Gd	0.649										
Gulf of Mexico											
n	11	11	11	11	11	11	11	8	10	11	
a	4	3	2	13	3	12	4	5	6	10	6.200
R_S	2.379	2.107	1.933	5.094	2.247	5.143	3.112	3.712	2.942	4.827	-

as	116-126	137-143	231-243	198-244	140-152	126-168	230-239	134-146	187-203	365-393	
H_E	0.502	0.437	0.455	0.931	0.558	0.939	0.710	0.808	0.579	0.913	0.651
H_O	0.364	0.364	0.636	0.909	0.636	0.909	0.727	0.875	0.500	1.000	0.692
HW	0.258	0.605	0.480	0.768	0.726	0.282	1.000	0.432	0.402	0.549	0.603
r	0.058	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.073	0.000	0.017
Overall Gd	-										

Brazil											
n	6	4	6	5	6	3	6	3	5	6	
a	5	3	3	8	2	4	5	3	5	9	4.700
R_S	3.924	2.5	2.741	5.167	1.998	4.000	3.924	3.000	3.400	5.318	-
as	116-128	137-145	231-243	206-244	150-152	130-142	230-242	134-140	179-199	359-399	
H_E	0.833	0.464	0.667	0.933	0.545	0.867	0.833	0.733	0.667	0.955	0.669
H_O	0.333	0.250	0.333	1.000	0.333	0.333	0.500	0.667	0.600	0.833	0.518
HW	0.040*	0.142	0.134	1.000	0.481	0.066	0.192	1.000	0.619	0.289	0.111
r	0.255	0.001	0.194	0.000	0.111	0.222	0.157	0.000	0.000	0.000	0.105
Overall Gd	-										

South Africa											
n	70	70	69	68	70	70	70	67	69	65	
a	9	4	5	26	6	13	7	8	12	16	10.600
R_S	3.319	2.532	1.959	5.283	1.674	4.120	2.509	3.356	3.220	4.924	9.037
as	116-132	137-143	231-245	198-260	124-156	124-162	227-245	125-146	179-215	359-389	
H_E	0.712	0.584	0.330	0.947	0.225	0.815	0.496	0.741	0.662	0.919	0.639
H_O	0.786	0.500	0.275	0.794	0.214	0.457	0.471	0.642	0.710	0.631	0.548
HW	0.476	0.503	0.002**	0.000**	0.120	0.000**	0.151	0.196	0.219	0.000**	0.000**
r	0.000	0.049	0.069	0.066	0.039	0.199	0.038	0.132	0.000	0.149	0.074

Overall Gd	0.644										
West Australia											
n	99	98	95	94	98	98	98	98	97	98	
a	8	5	7	32	8	19	6	7	12	19	12.300
R_S	3.491	2.696	2.033	5.365	1.99	4.217	1.979	3.237	3.141	4.891	9.171
as	116-130	137-145	225-245	196-264	138-156	116-168	227-242	125-143	171-231	355-397	
H_E	0.741	0.616	0.435	0.954	0.332	0.834	0.331	0.720	0.701	0.915	0.655
H_O	0.768	0.694	0.495	0.840	0.316	0.408	0.286	0.653	0.814	0.602	0.588
HW	0.835	0.533	0.000**	0.000**	0.216	0.000**	0.075	0.140	0.010*	0.000**	0.000**
r	0.000	0.000	0.017	0.051	0.031	0.227	0.061	0.000	0.000	0.158	0.055
Overall Gd	0.658										
East Australia											
n	84	82	78	84	84	82	76	80	83	80	
a	9	5	4	30	7	18	7	8	15	18	12.100
R_S	3.529	2.930	1.691	5.349	1.879	4.561	2.627	3.434	3.345	4.975	9.432
as	116-132	137-145	231-245	196-260	116-156	128-168	227-245	125-146	179-241	355-399	
H_E	0.756	0.664	0.251	0.953	0.298	0.875	0.570	0.748	0.692	0.924	0.669
H_O	0.762	0.622	0.269	0.929	0.321	0.561	0.342	0.750	0.627	0.900	0.608
HW	0.086	0.958	0.057	0.234	0.229	0.000**	0.000**	0.339	0.063*	0.123	0.000**
r	0.000	0.017	0.000	0.000	0.000	0.167	0.138	0.098	0.052	0.000	0.047
Overall Gd	0.674										
Indonesia											
n	16	15	17	17	17	17	15	16	14	12	
a	6	4	4	21	7	13	3	7	7	12	8.400

R_S	3.492	3.091	2.745	5.471	3.617	4.522	2.275	3.480	3.630	5.170	-
as	116-132	137-143	231-245	196-268	116-150	128-162	233-239	125-146	179-211	365-389	
H_E	0.738	0.701	0.585	0.963	0.766	0.877	0.476	0.742	0.751	0.942	0.731
H_O	0.813	0.733	0.353	0.765	0.647	0.882	0.467	0.563	0.429	0.750	0.643
HW	0.943	0.701	0.001**	0.002**	0.016**	0.679	0.770	0.054	0.035**	0.087	0.000**
r	0.000	0.000	0.156	0.083	0.057	0.013	0.001	0.000	0.184	0.075	0.057
Overall Gd	-										

North Australia											
n	33	31	30	33	33	33	32	32	29	29	
a	7	4	4	23	8	13	4	8	10	16	9.700
R_S	3.634	2.573	2.377	5.318	2.611	4.416	2.466	3.513	3.823	4.934	9.514
as	116-132	137-143	231-245	198-268	116-156	116-158	230-239	125-146	179-203	357-397	
H_E	0.772	0.577	0.466	0.951	0.503	0.864	0.537	0.754	0.791	0.920	0.702
H_O	0.818	0.581	0.233	0.879	0.394	0.515	0.563	0.719	0.828	0.828	0.636
HW	0.879	0.876	0.000**	0.074	0.004**	0.000**	0.307	0.772	0.484	0.174	0.001**
r	0.000	0.006	0.168	0.010	0.023	0.183	0.000	0.013	0.016	0.039	0.046
Overall Gd	0.715										

Overall										
r	0.031	0.012	0.070	0.031	0.026	0.110	0.040	0.032	0.034	0.049
H_E	0.647	0.498	0.364	0.839	0.379	0.763	0.473	0.639	0.602	0.778
H_O	0.695	0.535	0.334	0.895	0.355	0.621	0.437	0.653	0.706	0.758
HW	0.415	0.914	0.000**	0.000**	0.006**	0.000**	0.000**	0.170	0.019*	0.000**

*Indicates significant values, $P < 0.05$.

**Indicates significant values after sequential Bonferroni correction.

Tests for null alleles (FreeNa), indicated that nine of the ten surveyed loci (all except Cli108) demonstrated moderate levels of null alleles across more than one of the *a priori* defined populations. The frequency of null alleles exceeded 15% at six of the surveyed loci (Cli-107, Cpe-242, Cpe-421, Pgla-1, Pgla-5, and Ct-06) within a least one surveyed population. Of those six markers possessing a moderately high frequency of null alleles (15 – 25%), many also demonstrated a significant deviation from Hardy-Weinberg expectations within the surveyed population. To examine the potential effect of null alleles on population structure estimates, I utilized FreeNa's ENA (Excluding Null Alleles) correction method on the data set. This correction failed to significantly alter pairwise F_{ST} estimates from the previously estimated uncorrected values, and in fact were generally slightly higher (Figure 1); thus, the more conservative uncorrected F_{ST} values generated by ARLEQUIN are reported below. No evidence of linkage disequilibrium was detected across pairwise locus comparisons after sequential Bonferroni correction (α = 0.05/295).

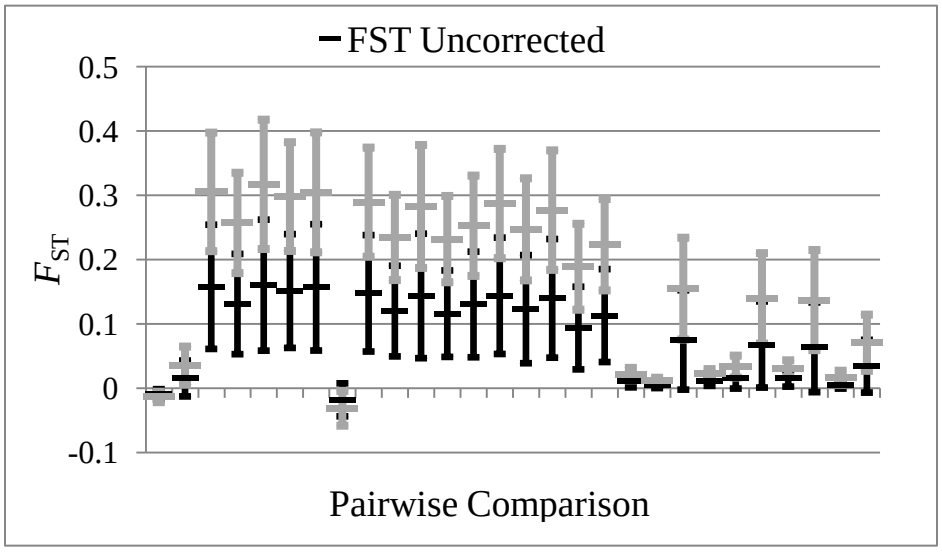

Figure1. Comparison of uncorrected pairwise F_{ST} with null-corrected (by FreeNa) F_{ST} values.

The Kruskal-Wallis one-way analysis of variance revealed no significant differences among populations with respect to *Gd* or R_S (Table 3). Power analysis, as implemented in POWSIM, revealed that the 10 microsatellite markers used in this study, in conjunction with the sampling scheme, was able to detect a true differentiation of F_{ST} = 0.003 with a 90% (Chi-square) and 100% (Fisher's exact test) probability. Alpha error of was estimated at 0.05. ML-Relate identified 17 pairs of highly related individuals (i.e., full-siblings, half-siblings, or parent-offspring relationships) within *a priori* defined populations: US East Coast, 6; South Africa, 1; Western Australia, 7; Eastern Australia, 3. *Post-hoc* tests of genetic differentiation revealed that the presence of related pairs of individuals did not significantly alter F_{ST} estimates (as estimated by ARLEQUIN), as F_{ST} estimates generated excluding of one of the two individuals from each 'related pair', were highly consistent. Given such negligible differences in F_{ST} estimates, the results reported herein include all genotyped individuals.

Population-level Subdivision

Overall, F_{ST} and D_{est} statistics identified strong genetic divergence between the western Atlantic and Indo-Pacific collections (F_{ST} = 0.054–0.151; D_{est} = 0.237-0.364), as well as less pronounced, but significant, substructure among the Indo-Pacific collections (F_{ST} = 0.009-0.047*;* D_{est} = 0.019-0.083). Overall *F*-statistics identified the presence of at least six genetically distinct populations across the dusky shark's global distribution: western North Atlantic (including US East Coast, Gulf of Mexico, and Brazil), South Africa, Western Australia, Northern Australia, Eastern Australia, and Indonesia; however, only nominally significant differences (F_{ST}) delineated Northern Australia and Eastern

Australia (Table 4). Concordant with *F*-statistics, D_{est} statistics failed to detect any significant differences between Northern Australian and Eastern Australian collections.

Table 4. Dusky shark population pairwise values of microsatellite F_{ST} (lower triangular matrix) and Jost's D (upper triangular matrix). USEC: US East Coast, GOM: Gulf of Mexico, BRA: Brazil, SAF: South Africa, WAU: Western Australia, EAU: Eastern Australia, IND: Indonesia, NAU: Northern Australia.

	USEC	GOM	BRA	SAF	WAU	EAU	IND	NAU
USEC	-	-0.005	0.05	0.314**	0.253**	0.329**	0.321**	0.364**
GOM	-0.009	-	-0.029	0.334**	0.259**	0.299**	0.329**	0.352**
BRA	-0.029	-0.037	-	0.265**	0.237**	0.284**	0.264**	0.331**
SAF	0.151**	0.139**	0.087**	-	0.023*	0.026*	0.083*	0.031*
WAU	0.122**	0.107**	0.06**	0.009**	-	0.027*	0.079*	0.044*
EAU	0.151**	0.128**	0.076**	0.005**	0.015**	-	0.046*	0.019
IND	0.134**	0.112**	0.054**	0.047**	0.041**	0.036**	-	0.061*
NAU	0.143**	0.122**	0.064**	0.012**	0.012**	0.005*	0.025**	-

*Indicates $P<0.05$

**Indicates significance after correction for multiple comparisons.

Strong genetic partitioning between the western Atlantic and Indian and Pacific collections was also confirmed by means of a hierarchical AMOVA, as highly significant population subdivision was found at all levels (Table 5).When collections were structured by ocean basin, group variance was only 8%, however, maximum group variance (~13%) was observed when samples were delineated into two global populations: western Atlantic and Indo-Pacific; although, variance within populations in groups was only ~1%.

Table 5. Results of the hierarchical AMOVA for microsatellite DNA. DF: degrees of freedom; VC: variance component; %V: percent of variance.

Comparison		**df**	**VC**	**%V**	**F_{ST}**	***P*-Value**
Among Groups	FCT	1	0.48	12.44	0.13	<0.00
Among Populations Within Groups	FSC	7	0.05	1.16	0.01	<0.00
Among Individuals Within Populations	FIS	391-409	0.35	9.12	0.11	<0.00
Within Individuals	FIT	400-418	2.98	77.26	0.23	<0.00

Partial mantel tests showed a significant correlation between geographic and genetic distance [$F_{ST}/(1-F_{ST})$] matrices ($r = 0.6013$, $P < 0.01$), demonstrating a significant global signal of isolation by distance. Hierarchical basin-level partial mantel tests, however, found no significant relationships between genetic and geographic distances within the Indo-Pacific (r = -0.43, $P = 0.79$), or western Atlantic collections (r = 0.06, $P = 0.66$) when analyzed separately.

Population-based PcoA showed strong divergence between western Atlantic and Indo-Pacific collection sites (Figure 2a). The first three coordinates explained 97.9% of the total variance, and coordinates 1 and 2 explained 79.7 and 13.1% of the total variation, respectively. Coordinate 1 separated the western Atlantic (including US East Coast, Gulf of Mexico, and Brazil) and Indo-Pacific (including: South Africa, Western Australia, Northern Australia, Indonesia, and Eastern Australia) collections, while coordinate 2 separated Brazil from Gulf of Mexico and US East Coast within the western Atlantic, and South Africa and Western Australia from Indonesia, Northern Australia, and Eastern Australia within the Indo-Pacific. Separate hierarchical analysis of the

western North Atlantic provided no evidence of further structure within the region (data not shown). However, separate analysis of the Indo-Pacific collections identified further genetic sub-structure within the region. The first three coordinates explained 98.6% of the total variance, and coordinates 1 and 2 explained 76.1 and 16.6% of the total variation, respectively. Coordinate 1 separated Western Australia, Eastern Australia, and South Africa from Northern Australia, and Indonesian collections, while coordinate 2 was unable to identify further substructure within regions (Figure 2b).

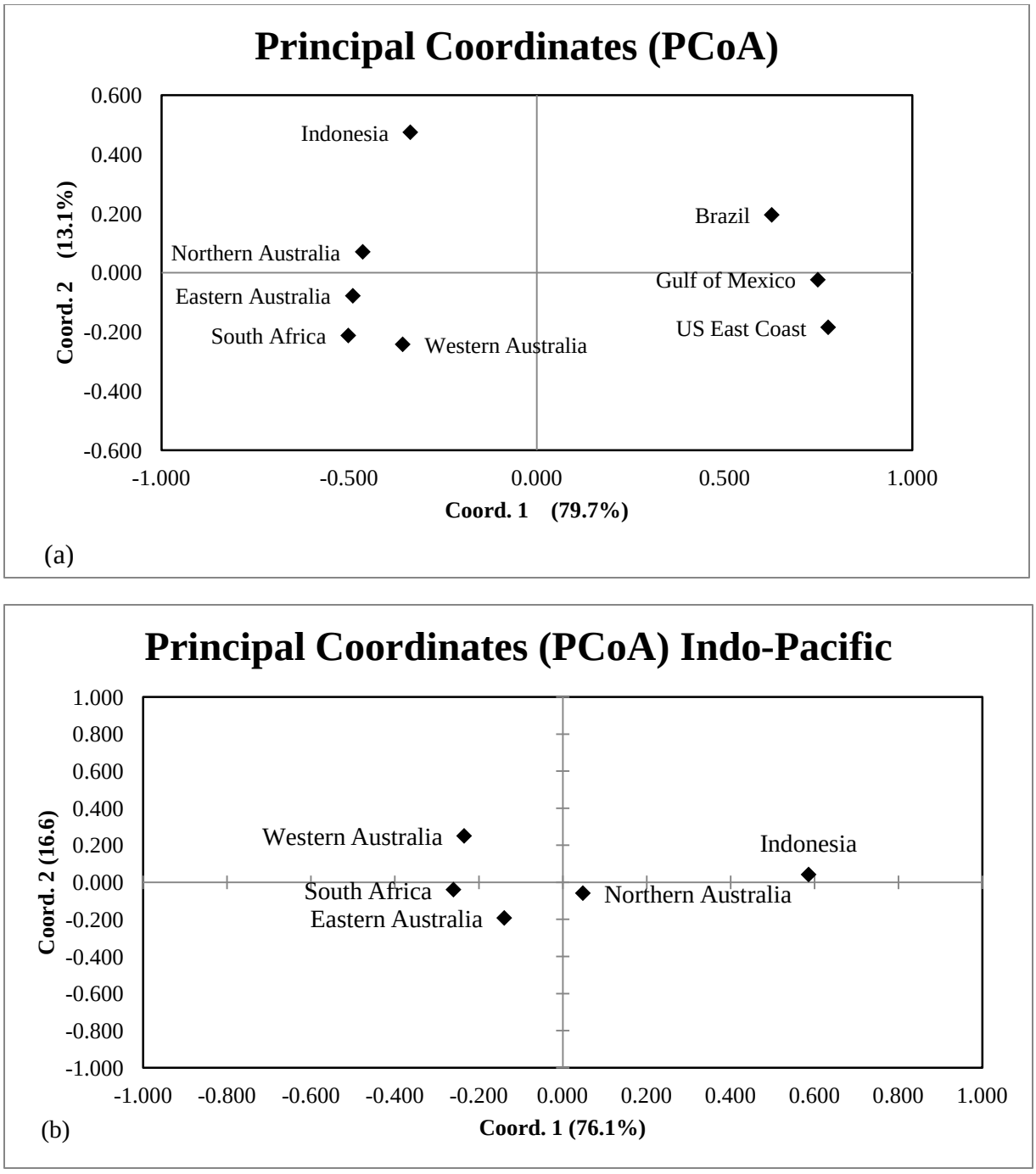

Figure 2. Population-level Principal Coordinate Analysis (PCoA) of dusky sharks (a) including all sample collections, and (b) hierarchical analysis of Indo-Pacific collections using GenAlEx 6.5

Individual level Subdivision

Results from both STRUCTURE analyses [i.e., with (Figure 3a) and without (Figure 3b) the *loc prior* model] were highly consistent; thus, results generated from only the *loc prior* model will be reported below. STRUCTURE identified $K = 3$ as the mostly likely number of genetically discrete populations within the global dataset, as mean ln likelihood values peaked at $K = 3$ [$\text{LnP}(K_3) = -13635.5$]. Evanno *et al.*'s (2005) parameter $\triangle K$, was more conservative, however, and identified $K = 2$ as the most probable state of nature ($\Delta K_2 = 210.6$). In fact, ΔK_2 was ~16 times higher than all other estimated ΔK values. At $K = 2$, membership coefficients revealed a strong genetic divergence between the western North Atlantic and Indo-Pacific collections (Figure 4a). Cluster 1 [$q = 0.716–0.994$; mean $q = 0.979$; standard deviation (std. dev.) = 0.038] largely comprised individuals from the US East Coast, Gulf of Mexico, and Brazilian collection sites, whereas Cluster 2 ($q = 0.643– 0.907$; mean $q = 0.873$; std. dev. = 0.045) included individuals from South Africa, Western Australia, Indonesia, Northern Australia, and Eastern Australia. One Brazilian individual possessed mixed ancestry ($q <$ 0.6 to either cluster). At $K = 3$, membership coefficients also suggested strong divergence among the western Atlantic, and Indo-Pacific collections; however, STRUCTURE revealed potential substructure within the Indo-Pacific as well :Cluster 1 ($q = 0.685$-987; mean $q = 0.966$; std. dev. = 0.041) mostly comprised individuals captured within the western Atlantic (US East Coast, Gulf of Mexico, and Brazil), Cluster 2 largely comprised individuals from South Africa, Western Australia, Eastern Australia, and Northern Australia ($q = 0.611$-0.962; mean $q = 0.872$; std. dev. = 0.072), and lastly Cluster 3 contained only a small subset of individuals from the entire dataset, as only ten

individuals were partitioned into this cluster, and all were collected from Indonesian waters (q = 0.602 -0.693; mean q = 0.645; std. dev. = 0.026) (Figure 4b). Interestingly, at K=3 a total of 11 individuals demonstrated mixed ancestry as they did not show strong assignment (q>0.600) to any of the three resolved clusters (Table 6). Hierarchical basin-level STRUCTURE analyses of the western Atlantic provided no evidence of substructure (data not shown). However, hierarchical basin-level STRUCTURE analyses of the Indo-Pacific collections indicated K = 2 as the mostly likely number of genetically discrete populations, as mean ln likelihood and ΔK values peaked at K = 2 [LnP(K_2) = -10087.6; ΔK_2 =5.621]. At K=2 membership coefficients reflect the isolation of Indonesian samples along with 18 northern Australian, 4 South African, 2 western Australian, and 19 eastern Australian samples in Cluster 1 (q = 0.604-0.934); mean q = 0.755; std. dev. = 0.104); Cluster 2 (q =0.504-0.967); mean q = 0.785; std. dev. = 0.126) contained all remaining Indo-Pacific samples with 32 individuals demonstrating mixed ancestry (Figure 4c).

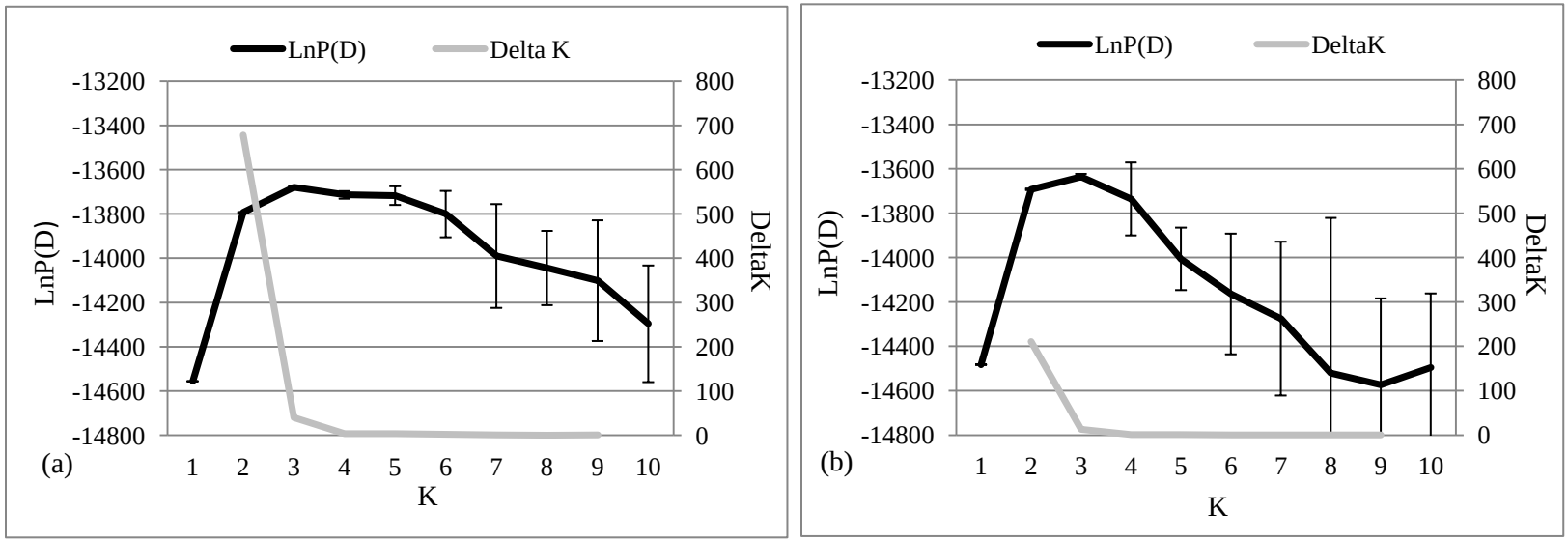

Figure 3. (a) (left vertical axis) Structure (Pritchard *et al.* 2000) output of the estimated Ln Probability of Data (Pr(X|K)) (± std. dev.) vs. cluster number (*K*) for the 10 globally distributed dusky shark sampling locations across 10 independent runs (100 000 burn-in, 300 000 MCMC iterations) assuming correlated allele frequencies, admixture and default settings; (right vertical axis) estimates of Evanno *et al.*'s (2005) parameter Δ*K* vs. cluster number (*K*); (b) (left vertical axis) Structure (Pritchard *et al.* 2000) output of the estimated Ln Probability of Data (Pr(X|K)) (± std. dev.) vs. cluster number (*K*) for the 10 globally distributed dusky shark sampling locations across 10 independent runs (100 000 burn-in, 300 000 MCMC iterations) assuming Hubisz *et al.*'s (2009)(2009) model *loc prior* and default settings; (right vertical axis) estimates of Evanno *et al.*'s (2005) parameter Δ*K* vs. cluster number (*K*)

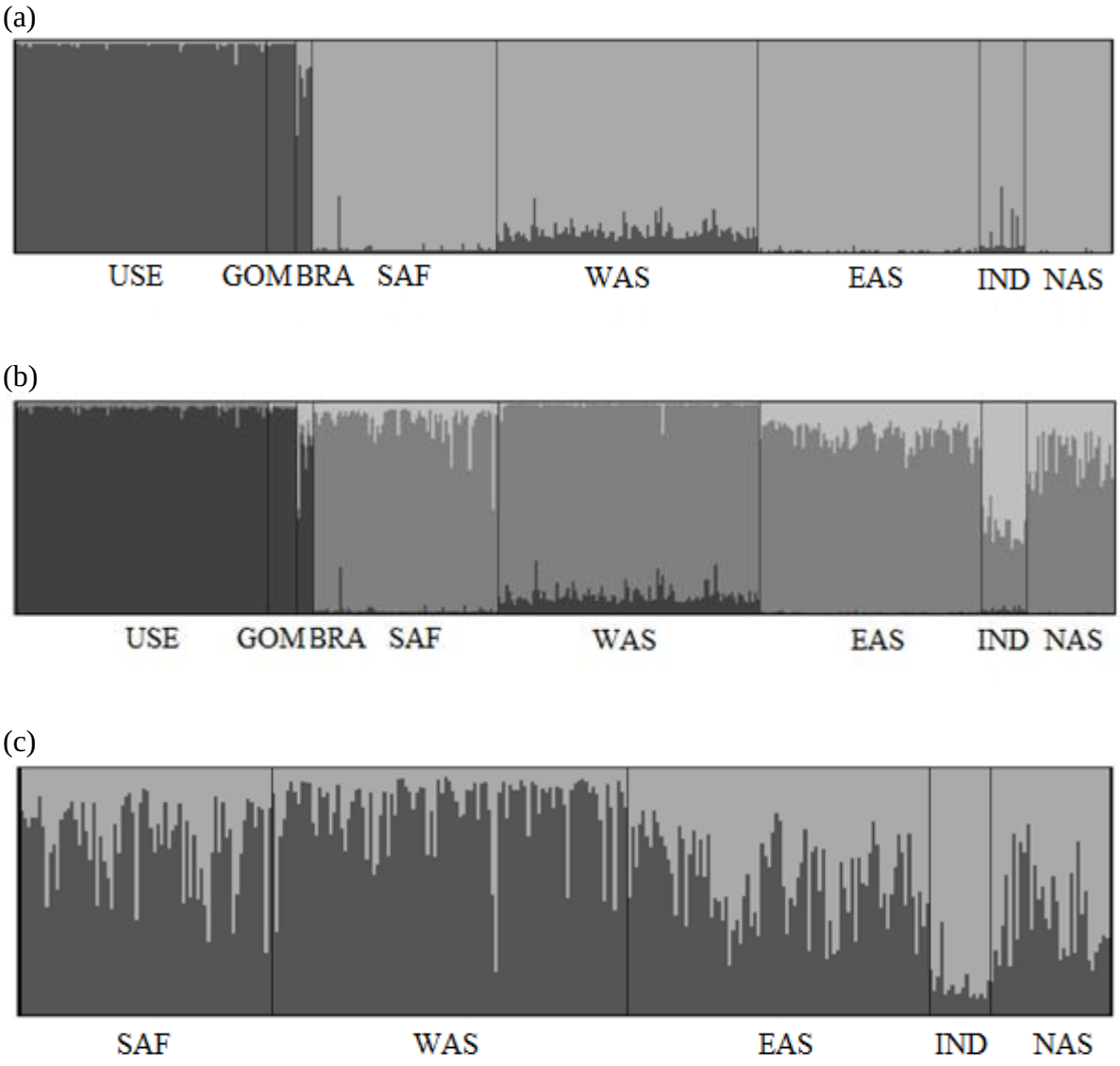

Figure 4. DISTRUCT v1.1 (Rosenberg 2004) plot of STRUCTURE results assuming Hubisz *et al.* (2009) *locprior* model assuming correlated allele frequencies and admixture, reporting proportional membership (*q*) of dusky sharks at (a) $K = 2$, (b) $K = 3$ and (c) within Indo-Pacific collections only. USE: US East Coast; GOM: Gulf of Mexico; SAF: South Africa; WAS: Western Australia; EAS: Eastern Australia; IND: Indonesia; NAS: Northern Australia.

Table 6. STRUCTURE assignments of *C. obscurus* individuals at *K*=3.

			q > 0.6		q < 0.6
Sampling location	*n*	Cluster 1	Cluster 2	Cluster 3	mixed ancestry
US East Coast	95	95			
Gulf of Mexico	11	11			
Brazil	6	5			1
South Africa	70		69		1
Western Australia	99		99		
Eastern Australia	84		84		
Northern Australia	33		31		2
Indonesia	17			10	7

Results from the program FLOCK provided similar results to those of STRUCTURE and suggested the presence of two or more genetically discrete populations. FLOCK identified a plateau of mean LLOD scores at $K = 2$, which satisfied stopping condition 2, thereby identifying the presence of two or more genetic partitions. Individual-based results at $K = 2$ delineated individuals into largely a western Atlantic partition, which included individuals from the US East Coast, Gulf of Mexico, and Brazilian collections, and a second partition, largely comprising individuals collected from within the Indo-Pacific, which consisted of South African, Indonesian, and all of the Australian samples (Table 7). Nine individuals were identified as possible migrants (i.e., migration between ocean basins) or as having mixed ancestry. Interestingly, the *C. obscurus* individuals identified as potential migrants (or as having mixed ancestry) were not concordant across analyses. For example, at $K = 2$, FLOCK and STRUCTURE each identified potential migrants (nine and one, respectively); however, the single Brazilian

individual identified as a potential migrant by STRUCTURE, was not one of the nine individuals recognized by FLOCK as at minimum having mixed ancestry.

Table 7. Number of dusky sharks allocated to *K*=2 reference groups by the program FLOCK.

		Allocated to	
Sampling location	*n*	Atlantic	Indo-pacific
US East Coast	95	92	3*
Gulf of Mexico	11	11	
Brazil	6	5	1*
South Africa	70	1*	69
Western Australia	99	3*	96
Indonesia	17	1*	16
Northern Australia	33		33
Eastern Australia	84		84

*Indicates possible migrants identified by FLOCK

GenAlEx's individual-based PcoA provided results which appear to be consistent with the STRUCTURE and FLOCK analyses and similarly identified two genetically differentiated populations: western Atlantic, and Indo-Pacific (Figure 5); however, the first three coordinates (12.6%, 4.6%, 4.1%, respectively) explained very little of the total variance (21.3%) found in the dataset.. Coordinate 1 separated the western Atlantic (including US East Coast, Gulf of Mexico, and Brazil) and Indo-Pacific (including: South Africa, Western Australia, Northern Australia, Indonesia, and Eastern Australia) collections, with slight overlap between the two groups. Coordinate 2 revealed no further substructure, as individuals from distinct collection sites showed no further clustering and

substantial overlap. Separate hierarchical analysis of the western North Atlantic and Indo-Pacific provided no evidence of further structure within these regions (data not shown).

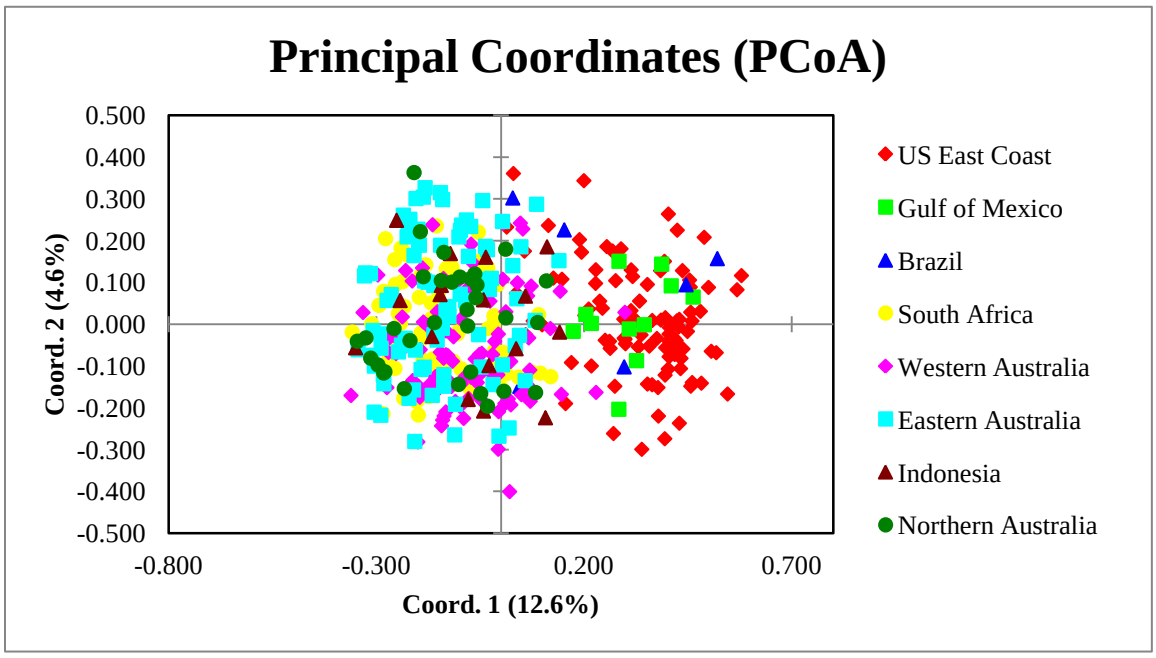

Figure 5. Individual-level Principal Coordinate Analysis (PCoA) of dusky sharks using GenAlEx 6.5

Contemporary and Historical Demographic History of Species

BOTTLENECK failed to identify a significant heterozygote excess in any of the surveyed populations. In contrast, the *M*-ratio test identified a reduction in population size ($M < M_c$) within a single *a priori* defined population (North Australia) at a θ of 0.01 and 0.1 (Table 8). The *k*- & g-tests largely inferred *C. obscurus* population sizes to be relatively stable over recent time-scales, with a single signal of recent expansion (within

5.1*Ne* generations) found within the South African collected, as indicated by a significant *k* test (Table 8). Some evidence of historical population declines was found by MSVAR across all surveyed populations (Table 9); however, population size estimates (N_0 and N_1) exhibited overlapping credibility intervals for all populations indicating no significant changes in population size over time.

Table 8. Tests for dusky shark demographic fluctuation under bottleneck or expanstion calculated using BOTTLENECK, *M*-ratio, and KGTESTS.

	Bottleneck		*M*-ratio ($M < M_c$)				KG test		
	Wilcoxon test for Het. Excess		Theta						
Sampling location	tpm	smm	0.01	0.1	1	10	*k*-test (*p*-value)	*g*-test	*g*-test threshold
US East Coast	0.98	0.99	FALSE	FALSE	FALSE	FALSE	1	1.545	0.18
South Africa	0.99	0.99	FALSE	FALSE	FALSE	FALSE	0.04*	3.57	0.18
Western Australia	0.99	0.99	FALSE	FALSE	FALSE	FALSE	0.34	0.9	0.18
Eastern Australia	0.99	0.99	FALSE	FALSE	FALSE	FALSE	0.8	1.35	0.17
Northern Australia	0.95	0.99	**TRUE**	**TRUE**	FALSE	FALSE	0.93	2.14	0.17

*Indicates $P<0.05$

Table 9. Bayesian estimation of demographic parameters using Log scale estimates of ancestral (N_1) and current (N_0) effective population sizes, time in years since decline (t_f), and mutation rate using MSVAR and the corresponding 95% credibility interval.

Location	Log_{10} N_0	Log_{10} N_1	Log_{10} t_f	Log_{10} μ
US East Coast	3.451 (2.409-4.281)	4.438 (3.774-5.188)	4.688 (3.079-5.95)	-3.554 (-3.959-3.151)
South Africa	4.008 (3.036-7.491)	4.167 (3.174-5.393)	4.904 (1.897-7.999)	-3.539 (-3.943-3.133)
Western Australia	3.566 (2.590-4.4)	4.587 (3.888-5.288)	4.89 (3.397-6.202)	-3.546 (-3.946-3.145)
Eastern Australia	3.206 (1.462-4.350)	4.429 (3.835-5.089)	3.883 (2.194-5.313)	-3.574 (-3.980-3.167)
Northern Australia	3.18 (1.612-4.228)	4.528 (3.897-5.169)	4.023 (2.404-5.419)	-3.568 (-3.971-3.164)

[a]Results show the median value and 5–95% credbility intervals.

Geographic sourcing of market fins

The individual-based analyses STRUCTURE (ΔK) and FLOCK suggested that the global dataset most likely comprised only two, relatively discrete, homogeneous populations, which comprised: (i) the western Atlantic (US East Coast, Gulf of Mexico, and Brazil), and (ii) the Indo-Pacific (South Africa, Indonesia, and Australia). Thus, genetic assignment of the Hong Kong fins was performed assuming that each fin could be assigned to one of these two broad oceanic regions (i.e., western Atlantic, or Indo-Pacific). Fin assignments were largely concordant across methods and suggested that the majority of fins likely originated from animals captured within the western Atlantic (Table 10). A total of 15 of the 22 surveyed Hong Kong market fins were genetically assigned to the western Atlantic using all four assignment tests (STRUCTURE, FLOCK, SCAT, and ONCOR): 12 fins were assigned with high statistical confidence (assignment score or $q > 0.95$), while three were assigned with lower confidence (assignment score or $q > 0.60$). Conversely, four Hong Kong fins were assigned to Indo-Pacific waters via all four assignment techniques with a range of statistical confidence (assignment score or $q = 1.00 – 0.62$). In addition, three market derived fins showed inconsistent levels of assignment across statistical methods and were therefore considered to possess an 'ambiguous' assignment, as they were not confidently assigned to either Ocean basin.

Table 10. Fin assignment results by method and membership coefficient (q), assignment score, or probability. WA: Western Atlantic, IP: Indo-Pacific

		Assignment test:								Exclusion test:	
		Structure		Flock		Scat		ONCOR		GENECLASS	
	Individual	Assign to	*q*	Assign to	Score	Assign to	Score	Assign to	Score	Assign to	Probability
a											
	HKF097	WA	1.00	WA	1.00	WA	100.00	WA	1.00	**WA**, IP	0.31, 0.03
	HKF601	WA	1.00	WA	1.00	WA	100.00	WA	1.00	**WA**, IP	0.67, 0.13
	HKF603	WA	1.00	WA	1.00	WA	100.00	WA	1.00	**WA**, IP	0.81, 0.01
	HKF732	WA	1.00	WA	1.00	WA	100.00	WA	1.00	**WA**, IP	0.97, 0.24
	HKF600	WA	0.99	WA	1.00	WA	99.99	WA	1.00	**WA**, IP	0.08, 0.02
	HKF832	WA	0.84	WA	1.00	WA	99.99	WA	1.00	**WA**, IP	0.32, 0.07
	HKF597	WA	0.99	WA	1.00	WA	99.62	WA	1.00	**WA**, IP	0.17, 0.05
	HKF533	WA	0.98	WA	1.00	WA	99.50	WA	1.00	**WA**, IP	0.08, 0.05
	HKF439	WA	0.95	WA	1.00	WA	99.40	WA	1.00	**WA**, IP	0.02, 0.01
	HKF443	WA	0.97	WA	1.00	WA	99.36	WA	0.99	**WA**, IP	0.05, 0.03
	HKF444	WA	0.96	WA	1.00	WA	99.35	WA	0.99	**WA**, IP	0.08, 0.07
	HKF599	WA	0.87	WA	1.00	WA	99.06	WA	0.90	**WA**, IP	0.05, 0.04
	HKF437	WA	0.94	WA	1.00	WA	98.71	WA	0.98	WA, **IP**	0.07, 0.08
	HKF442	WA	0.95	WA	1.00	WA	97.28	WA	0.98	**WA**, IP	0.03, 0.02
	HKF602	WA	0.77	WA	1.00	WA	88.04	WA	0.61	WA, **IP**	0.05, 0.07
b											
	HKF596	IP	1.00	IP	0.98	IP	99.99	IP	1.00	**IP**	0.00, 0.14
	HKF018	IP	0.82	IP	0.87	IP	98.98	IP	1.00	**None**	0.00, 0.00
	HKF831	IP	0.91	IP	1.00	IP	96.22	IP	0.97	WA, **IP**	0.09, 0.36
	HKF367	IP	0.82	IP	0.81	IP	81.68	IP	0.62	WA, **IP**	0.06, 0.21
c											
	HKF440	IP	0.52	WA	1.00	WA	93.40	IP	1.00	**IP**	0.00, 0.01
	HKF833	IP	0.78	WA	1.00	IP	89.73	IP	0.87	WA, **IP**	0.01, 0.03
	HKF330	IP	0.92	IP	0.97	WA	70.56	IP	0.97	WA, **IP**	0.01, 0.06

[a] Individual fins assigned by all assignment programs to the Western Atlantic reference population.
[b] Individual fins assigned to the Indo-Pacific reference population.
[c] Individual fins with ambiguous assignments. None: fin sample excluded from both reference populations. Bold values indicate the population with highest probability of assignment using the exclusion test.

The Bayesian exclusion test, as implemented in the program GENECLASS, was only able to assign seven of the 22 Hong Kong fins to one of the two reference populations beyond the exclusion threshold of $P < 0.10$. A single Hong Kong fin (HKF018) was statistically excluded from both reference populations ($P = 0$), thereby suggesting that the geographic source population for this particular multilocus genotype was not sampled (or not included as a reference population).

Discussion

This study is the first to investigate the global population structure and genetic diversity of the dusky shark utilizing nuclear microsatellite DNA. Interestingly, despite the known high dispersal capacity of this species (Rogers *et al.* 2013b; Hussey *et al.* 2009; Kohler *et al.* 1998b), indications of restricted gene flow (both male and female) were found at unexpected levels, as evidence for at least six globally distributed genetic MUs was found. In addition, the identification of high levels of genetic population structure, coupled with a large utilized sample set, allowed for the first application of individual-based genetic assignment methods to source Hong Kong market derived dusky shark fins to their most likely geographic source population (i.e., original capture location).

Genetic diversity and population structure

Population- and individual-level analyses both suggested the presence of multiple global genetic MUs as well as strong genetic partitioning between the western Atlantic and Indo-Pacific Ocean basins. Within western Atlantic waters, microsatellite DNA revealed high levels of connectivity amongst dusky shark collection sites (US East Coast, Gulf of Mexico, and Brazil) demonstrating mixed concordance with patterns of differentiation

previously identified using mitochondrial DNA within the same surveyed regions (see Benavides *et al.* 2011). Akin to Benavides *et al.* (2011), negligible genetic differences between the US East Coast and Gulf of Mexico collections were found; however, in contrast to the present microsatellite DNA analyses, Benavides *et al.* (2011) reported genetic differences between dusky sharks from the western North Atlantic and those from the western South Atlantic (Brazil). Unfortunately, Benavides *et al.* (2011) note that the provenance of their Brazilian samples could not be confirmed, as they were collected from a Hong Kong fin market rather than from a wild source population. Thus, any inferred differentiation between the western Atlantic hemispheric collections found by Benavides *et al.* (2011) is largely circumspect. In the present study, while no genetic differentiation was found between western North and South Atlantic collections, it is important to note that inferences regarding the level of connectivity between these collections were limited due to a low Brazilian sample size ($n = 6$).

Similar to several previous mtDNA surveys, highly significant Indo-Pacific genetic population substructure was observed via nuclear microsatellite DNA. As in the present study, Benavides *et al.*'s (2011) mtCR survey revealed strong genetic partitioning between South African and western Australian collection sites, suggesting that connectivity between both male and female dusky sharks inhabiting the eastern and western peripheral waters of the Indian Ocean is limited [see Benavides *et al.* (2011)]. Within Australian waters, this same mtCR survey found a high degree of gene flow and haplotype sharing between eastern and western Australian dusky shark collections ($\Phi_{ST} = 0.015$, $P > 0.17$) (Benavides *et al.* 2011); however, their ability to detect fine-scale differentiation within Australian waters was likely limited by the use of low sample sizes,

particularly within the waters of eastern Australia (n = 16). In contrast, Geraghty *et al.* (2014) surveyed largely these same eastern and western coastal regions utilizing the mitochondrial protein coding locus ND4, as well as much larger sample set (New South Wales, n = 301; western Australia, n = 57), and found statistically significant differentiation (Φ_{ST} = 0.04437, P < 0.008) between these two distal coasts, suggesting mixing of dusky sharks between Australian coastal regions may be at least somewhat limited. Microsatellite DNA analyses confirmed Geraghty *et al.*'s (2014) findings, as highly significant genetic differentiation (Table 4) was found delineating eastern and western Australian collections.

In the present study, microsatellite DNA suggested high genetic partitioning across the Indo-Australian archipelago, as highly statistically significant genetic differentiation was found between those samples collected from Indonesia and all three Australian collection sites (F_{ST} = 0.025-0.041; P < 0.01). In fact, despite a much lower ability to resolve genetic structure compared to traditional pairwise metrics (see Orozco-terWengel *et al.* 2011; Waples and Gaggiotti 2006; Jones and Wang 2012; Latch *et al.* 2006), results produced by the individual-based multi-locus clustering program STRUCTURE suggested the presence of two distinct genetic partitions within Indo-Pacific waters: one comprising the bulk of those samples collected from the waters of Indonesia, and one largely comprising individuals from the remainder of the dusky shark's surveyed Indo-Pacific distribution (Figure 4c). Unfortunately, the exact capture location of the highly differentiated Indonesian samples remains unknown, as they were collected from the Tanjung Luar market located in eastern Lombok, West Nusa Tenggara, and not sampled directly from a wild source location (Geraghty *et al.* 2014).

However, given that the market is predominantly supplied by the local artisanal fishery (Thia-Eng *et al.* 1997), these samples likely originated from waters in close proximity to eastern Lombok. Interestingly, Geraghty *et al.*'s (2014) ND4 survey included many of these same samples (Indonesia, $n = 16$; East Australia, $n = 68$; North Australia, $n = 33$), yet the western and northern Australian samples were found to be only nominally differentiated, rather than significantly differentiated after adjustment for multiple comparison tests, from Indonesian samples via F_{ST} but not via Φ_{ST} ($F_{ST} = 0.07440 – 0.13925$, $P \leq 0.05$; $\Phi_{ST} = 0.02476 – 0.03010$, $P > 0.05$). In contrast to Geraghty *et al.* (2014), Ovenden *et al.* (2009) reported nearly three times higher levels of differentiation between collections from Indonesia and Australia; however, significant differentiation was once again only found between Indonesian and western Australian samples ($\Phi_{ST} = 0.191$, $P < 0.05$). No significant differences were found between eastern Australian dusky sharks and those from Indonesia, however, only a few individuals were surveyed from eastern Australia (n = 7).

The highly significant nuclear genetic differentiation found among Indo-Pacific collection sites coupled with the absence of a signal of isolation by distance, suggests reduced gene flow and the potential presence of at least some form of barrier (i.e., physical or biological) to dispersal. The dusky shark is described as a coastal- pelagic species, as numerous studies have documented this species partaking in long-distance migrations (1323 - 2736km) (Rogers *et al.* 2013b; Hussey *et al.* 2009; Kohler *et al.* 1998b). Consequently, such fine-scale genetic population structure, as revealed by microsatellite DNA was unexpected, especially among the Australian collection sites (western, northern, and eastern), which were positioned along a contiguous coastline, and

in some cases, separated by only relatively short distances (~2200 km). Open ocean expanses or deep waters have been suggested as a potential barrier to gene flow for numerous shark species (Duncan *et al.* 2006; Keeney and Heist 2006; Schultz *et al.* 2008; Benavides *et al.* 2011; Whitney *et al.* 2012; Karl *et al.* 2012), and to date, no evidence of open ocean long distance migrations have been documented for the dusky shark, which suggests that deep waters may potentially serve as a barrier to dispersal for this species as well. To explain the significant differentiation found between Indonesian and western Australian spot-tail shark (*C. sorrah*) collections, Ovenden *et al.* (2009) suggested that the Timor Trench within the waters of the Timor Sea, located between northwestern Australia and Timor Island, may at least partially serve as a barrier to dispersal for this coastal species, as the trench approaches a depth of ~3000m in some regions and may therefore discourage some animals from traversing these waters. Without knowledge of the exact capture location of the Indonesian samples used in the present study, the identification of the potential mechanisms restricting the dispersal of dusky sharks within this region remains speculative at best; however, the Timor Trench cannot be excluded as a potential mechanism limiting dispersal of dusky sharks within Indo-Australian waters.

With respect to the high levels of population structure found within Australian waters, few (if any) contemporary physical barriers to dispersal exist which may account for the level of genetic structure found in the current study. Furthermore, long distance migrations (~2700km) have been documented for this species within Australian waters (Rogers *et al.* 2013b) suggesting that contemporary mixing of some coastal populations does occur. For instance, Rogers *et al.* (2013b) documented the westward migrations of three dusky sharks originally captured within the Spencer Gulf, South Australia, using

pop-up satellite archival tags (PSAT). While all three individuals traveled a minimum of 1760 km, a single individual travelled > 2700 km to the coastal waters of Western Australia, suggesting the potential for extensive genetic connectivity; however, genetic sampling of *C. obscurus* within southern Australia is required to confirm gene flow between these areas. To account for the high levels of genetic differentiation observed between western and eastern Australian dusky shark populations using the mitochondrial locus ND4, Geraghty *et al.* (2014) suggested that sea-level and temperature changes during the Pleistocene glacial periods may have contributed to the historical isolation of dusky sharks inhabiting these regions. Furthermore, Geraghty *et al.* (2014) offered that if dusky sharks were restricted to Northern Australian waters (i.e. Southern waters became uninhabitable for dusky sharks) during glacial maxima, the formation of the Torres Strait land bridge during periods of sea-level minima may have potentially isolated eastern and western collections allowing for substantial genetic drift to occur between these populations. While these historical barriers have since resolved, the current genetic subdivision between these regions may be an artifact of this historical separation and may be maintained at contemporaneous time-scales by behavioral mechanisms, such as site-fidelity. Regional genetic sub-division in the absence of obvious physical barriers has been documented in many other shark species [white shark *Carcharodon carcharias* (Blower *et al.* 2012), bull shark *Carcharhinus leucas* (Karl *et al.* 2011), spot-tail shark *Carcharhinus sorrah* (Ovenden *et al.* 2009)] suggesting that some species, despite the potential for long distance migrations, may in fact possess relatively limited home ranges.

In many shark species, female reproductive philopatry, which is the tendency of a female to return to, or remain in, a home area, natal site, or other adopted region (Mayr

1963), is often employed to explain high levels of genetic population structure in the absence of physical barriers. Female philopatry typically consists of high mtDNA differentiation coupled with low nuclear differentiation. This pattern is influenced by the maternal inheritance of mtDNA and typically occurs when females return to their natal region for parturition while males are more prone to dispersal. However, much higher levels of differentiation among populations was observed via microsatellite DNA (present study) than in previous mtDNA (ND4 and mtCR) studies, suggesting that female philopatry is likely not the only cause of such fine-scale structure. *Post-hoc* nuclear-based tests for sex-biased dispersal were performed by examining differences in F_{IS}, F_{ST}, relatedness, mean assignment index, and variance of assignment indices between males and females (where sex data was available) using FSTAT; however, all of the above tests were non-significant (data not shown). In addition, no evidence of bias due to the inadvertent sampling of related individuals was detected. Thus, the higher nuclear-based genetic differentiation found in the present study could not be attributed to either the non-random sampling of individuals within collections, or sex-biased dispersal. Therefore, as a species, dusky sharks may exhibit substantial site fidelity or possess restricted home ranges, which may account for the significant regional genetic discontinuities detected within Australian waters rather than any contemporary physical barriers to dispersal.

Demographic History

Fishery dependent data from the western North Atlantic Ocean suggests that severe reductions in dusky shark abundance have occurred within the last 2-4 decades (Baum and Myers 2004; Musick *et al.* 1993; Myers *et al.* 2007). However, despite evidence of drastic declines, microsatellite DNA analyses (BOTTLENECK, *M*-Ratio, MSVAR)

suggested largely stable contemporary and historical effective population sizes within western Atlantic waters, as well as across all other collections. Although microsatellite DNA analyses failed to detect a signature of population decline, genetics-based inferences of the demographic history of species are often problematic, as statistical model violations (e.g., Hardy-Weinberg disequilibrium, mutation model) and poor parameterization may provide limited resolution using such methods (Williamson-Natesan 2005). Furthermore, the temporal and demographic sensitivities of bottleneck detection methods have been shown to be widely variable (Spear *et al.* 2006; Beebee and Rowe 2001; Williamson-Natesan 2005; Busch *et al.* 2007), suggesting that bottleneck inferences derived from genetic data should be viewed cautiously. In addition to the above statistical caveats, the dusky shark possesses an extremely long generation time [30-40 years (Cortés *et al.* 2006; SEDAR 2011)]; thus, it remains plausible that sufficient time has not yet passed for a recent (and severe) population decline to be detected (England *et al.* 2003; Weber *et al.* 2004; Lippe *et al.* 2006).

Geographic sourcing of market fins

While market-derived Hong Kong fins could not be assigned to a precise geographic location, the set of nuclear microsatellite markers and sampling regime used in the present study allowed for the successful assignment of most of the surveyed fins to a broad geographic region or Ocean basin. In fact, for many of the fins, concordant geographic assignments occurred across many of the varying analysis programs (Table 10). Such high levels of concordance among statistical assignment methods likely stems from the high level of differentiation between the western Atlantic and Indo-Pacific collections of dusky sharks (F_{ST} = 0.12 - 0.15), as this level of divergence exceeds

previously identified thresholds for accurate individual-based assignment (see Manel *et al.* 2002). Interestingly, of the surveyed Hong Kong dusky shark fins, the majority of individuals (15 of 22) were identified as having most likely originated from the western North Atlantic based on their multilocus genotype (Table 10). This high level of Ocean basin assignment bias was quite surprising, and may have a number of important ecological and biological consequences for dusky sharks. For instance, if a disproportionate number of dusky sharks exploited within the fin trade are caught within the waters of the western Atlantic, dusky sharks within this region may require additional conservation or management actions to ensure their persistence. While it is impossible at this time to assign these market fins to a more precise geographic location (i.e., western North vs South Atlantic), it is important to note that dusky sharks from the western North Atlantic are currently listed as 'Endangered' by the IUCN due to severe declines in abundance (Musick *et al.* 2009). Furthermore, within US Atlantic waters dusky sharks have been federally protected by the Fishery Management Plan for Atlantic tunas, swordfish, and sharks since 2000 (NMFS 1999). As geographic assignment of fins could only be made to broad spatial regions, it is impossible to conclude that illegal harvest of dusky sharks is occurring from within US waters. Additionally, based on genetic evidence from Benavides *et al.* (2011), and data from both conventional mark-recapture and satellite tracking studies (Hoffmayer *et al.* 2014; Hoffmayer *et al.* 2010; Kohler *et al.* 1998b), dusky sharks within the western Atlantic are highly mobile and show high connectivity across large areas. Combined, the above suggests that while US waters may offer some degree of protection for this highly overexploited species, it is likely that western Atlantic dusky sharks posses an extremely large home range and regularly travel

outside the boundaries of US waters. Thus, refuge within US federally protected waters affords them only minimal or intermittent protection.

Although the present study found a strong Ocean-basin bias with respect to the geographic assignment of market derived fins, it is important to take note of several key caveats: (1) that the Hong Kong fins collected by Clarke *et al.* (2006a) are likely not a true representation of the overall harvest and exploitation rates of dusky sharks in the fin trade. Clarke *et al.*'s (2006a) survey is simply a brief snapshot of the market dynamics occurring at the time when the fins were sampled (November 2000 to February 2002), rather than a long-term survey of the dusky shark fin trade; and (2) the basin-level assignment bias that was detected is also contingent on the assertion that the global distribution of dusky sharks has been adequately (and exhaustively) sampled to ensure that all potential source populations have been surveyed and are included as reference populations (Manel *et al.* 2002; Cornuet *et al.* 1999; Manel *et al.* 2005). If the true source population has not been sampled, many assignment tests will still assign an individual to a reference population, as the 'erroneous source' population is still the most likely population relative to all other surveyed populations. To circumvent this problem, exclusion tests (i.e., GENECLASS) may serve to help determine if the true source population has in fact been sampled. Unlike many other assignment programs, GENECLASS may reject any and all of the available reference populations as the most likely population of origin, when the true source has not been sampled, rendering it an invaluable assignment tool. Unfortunately, large areas of the global distribution of dusky sharks were not sampled in the present study, and results from the GENECLASS exclusion test also suggested that not all global source populations had been adequately

sampled. While six of the 15 western-Atlantic sourced fins exceeded GENECLASS' assignment threshold, the majority of fins failed to exceed this threshold. Thus, until the distribution of the dusky shark has been thoroughly genetically assessed, this disproportionate assignment of Hong Kong fins to the western Atlantic Ocean is highly conditional. Nevertheless, this study is the first to provide managers and policymakers with even a small amount of genetic data concerning trade activity occurring in critical conservation areas for the dusky shark or any other shark species. Interestingly, Benavides *et al.* (2011) determined via a simulated mixed stock analysis (MSA) that the level of mtCR variation and differentiation found within their study was sufficient to allow for sourcing of dusky sharks to one the three identified global MUs (western Atlantic, South Africa, and Australia). However, they acknowledged that a comprehensive survey of global stock structure was required to confirm the presence of distinct management units, and recommended the use of nuclear markers to aid sourcing efforts. Thus a combination of these two marker systems (mtDNA and nuclear microsatellites) may allow for increased precision in geographic assignment of market fins in future studies.

It is important to note, that for each method used to conduct population assignments, numerous inherent limitations exist. Many of the assignment methods used in the present study operate under the assumption that each of the reference populations included in the analysis are in both Hardy-Weinberg and linkage equilibrium (STRUCTURE, SCAT, & ONCOR) (Pritchard *et al.* 2000; Wasser *et al.* 2004; Kalinowski *et al.* 2007). Violation of these assumptions may introduce bias and/or cause erroneous assignments leading to inaccurate results (Manel *et al.* 2002). Cornuet *et al.*

(1999) investigated the effect of deviations from HWE using likelihood assignment methods and found little variation in assignment scores when allele frequencies within reference populations deviated from HWE (as in the present study) . Notably, the iterative allocation procedure implemented in FLOCK does not assume Hardy-Weinberg equilibrium within populations, and 19 of FLOCK's fin assignments were concordant with STRUCTURE, ONCOR, and SCAT results.

Concluding Remarks

'Wildlife crime' is a global multi-billion dollar industry and is defined as the illegal trade, transport, or harvest of animals or their derivatives in violation of current laws and treaties (Wyler and Sheikh 2008). For many species, harvest may be illicit within certain regions or physical boundaries; thus, identifying the wild, geographic source of an animal product may be essential to identify illegal possession or trade activity (Ogden *et al.* 2009). However, the enforcement of region specific wildlife protection laws has been constrained when physically distinguishing characteristics between legal and illegally obtained specimens is absent. Fortunately, recent DNA technologies have allowed for accurate and robust species identification in the absence of morphologically distinguishing characteristics (Clarke *et al.* 2006b; Clarke *et al.* 2006a; Hoelzel 2001; Moore *et al.* 2003; Pank *et al.* 2001; Magnussen *et al.* 2007; Shivji *et al.* 2005) and in some cases can also be applied to identify the origin of market derived or seized wildlife products (Sanders *et al.* 2008; Wasser *et al.* 2004; Velo-Antón *et al.* 2007; Frantz *et al.* 2006; Renshaw *et al.* 2006). For instance, Clarke *et al.* (2006a) used a species-specific PCR assay to determine species composition and proportion in a Hong Kong shark fin markets; and Wasser *et al.* (2004) applied microsatellite-based assignment tests to

identify the origin of seized African elephant tusks. Although DNA technologies have many applications to wildlife conservation and law enforcement, population assignment tests often require high levels of genetic population structure within the species of interest to identify distinct MUs and are often contingent upon the comprehensive sampling of MUs.

The global patterns of population genetic structure inferred using 10 nuclear microsatellite loci were broadly similar to previous mtDNA results (Benavides *et al.* 2011) demonstrating the strong genetic differentiation found between the western Atlantic and Indo-Pacific dusky sharks, which is also highly consistent with many other species of shark (Castro *et al.* 2007; Duncan *et al.* 2006; Keeney and Heist 2006). In contrast, the regional genetic population structure observed within Australia's coastal waters was largely unexpected; however, such high levels of differentiation are consistent with mounting evidence that some very large sharks may exhibit restricted dispersal or site fidelity across relatively small spatial scales due to a number of presumed known and unknown biological and physical factors (Blower *et al.* 2012; Geraghty *et al.* 2013; Tillett *et al.* 2012b; Tillett *et al.* 2012a).

The mito-nuclear discordance, with respect to the level of genetic differentiation detected between marker types, was also surprising. Such discordance has been described in other species within this genus [white sharks, *Carcharodon carcharias* (Blower *et al.* 2012); grey nurse shark, *Carcharias taurus* (Ahonen *et al.* 2009)]; however, few studies have reported higher levels of population structure using nuclear microsatellites than with mitochondrial DNA. In the absence of sex-biased dispersal, variation in sample size across studies likely contributed significantly to such discordance.

The detection of fine-scale population structure within the Indo-Pacific basin requires a localized management approach to ensure the sustainability and persistence of individual MUs. In fact, the concordance across studies and marker types (mtDNA vs microsatellite) differentiating Indonesian and Australian dusky sharks, underscores the need to manage these populations separately as they are likely demographically independent. Furthermore, based on the level of fine-scale genetic population structure detected within Indo-Pacific waters, the potential for numerous isolated populations exists across the dusky shark's global distribution, indicating that additional genetic surveys are required to properly address this species' genetic connectivity across its range. Future research directed at the genetic sampling of data-deficient oceanic regions and long term tagging or tracking are necessary to expand upon the current knowledge base for this species. From the present study, it is clear that genetic approaches to conservation and management may not only help to ensure the persistence of species, but also aid in identifying populations undergoing high levels of both illegal and legal overexploitation.

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